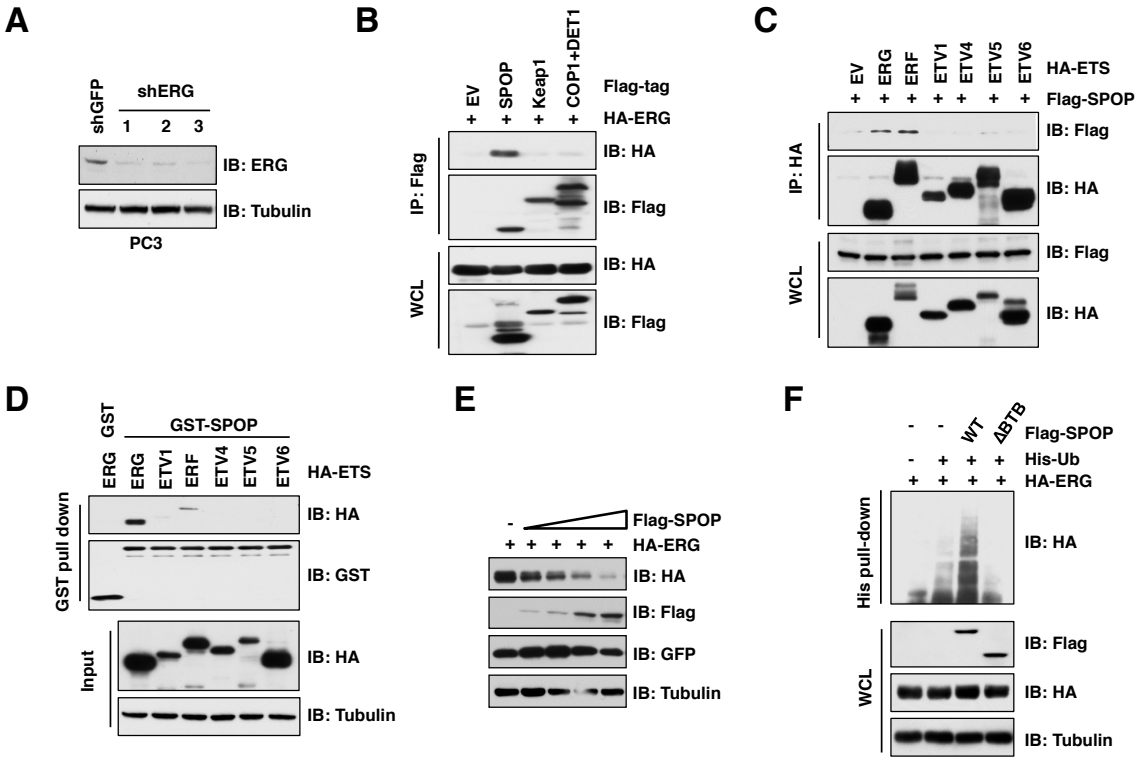

Figure S1. ERG specifically interacts with SPOP (related to Figure 1)

A. Immunoblot (IB) analysis of whole cell lysates (WCL) derived from PC3 cells infected with control (shGFP) and ERG (shERG) lentiviral shRNA vectors. The infected cells were selected with 1 μg/ml puromycin for 72 hours to eliminate the non-infected cells before harvesting.

B. IB analysis of WCL and immunoprecipitates (IP) derived from 293T cells transfected with HA-ERG and Flag-SPOP, Keap1, COP1 constructs. Thirty hours post-transfection, cells were treated with 10 μM MG132 for 10 hours before harvesting.

C. SPOP interacts specifically with ERG and ERF. IB analysis of WCL and IP derived from 293T cells transfected with Flag-SPOP and HA-tagged members of ETS family constructs. Thirty hours post-transfection, cells were treated with 10 μM MG132 for 10 hours before harvesting.

D. GST-pull down assay showed that ERG and ERF, but not ETV1, ETV4 and ETV5, interact with SPOP.

E. IB analysis of WCL derived from 293T cells transfected with ERG and increased amount of Flag-SPOP-WT.

F. *In vivo* ubiquitination assay analysis of WCL and His pull-down of PC3 cells transfected with the indicated plasmids to demonstrate that only WT-SPOP, but not ΔBTB-SPOP, can promote ERG ubiquitination. Thirty hours after transfection, cells were treated with 20 μM MG132 for 6 hours before cell collection. Ni-NTA, nickel-nitrilotriacetic acid.

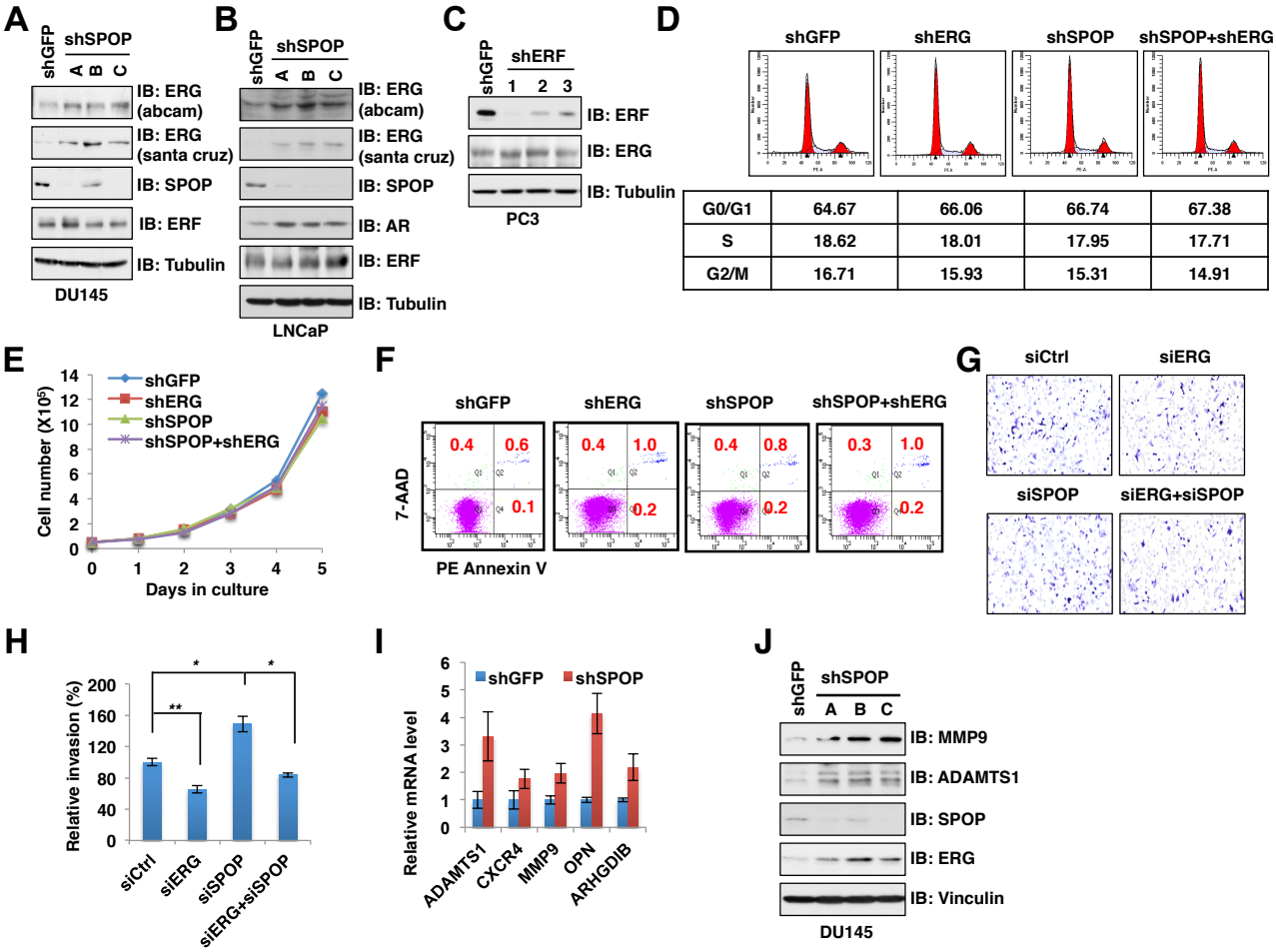
A
shGFP
shSPOP
A B C
IB: ERG (abcam)
IB: ERG (santa cruz)
IB: SPOP
IB: ERF
IB: Tubulin
DU145
B
shGFP
shSPOP
A B C
IB: ERG (abcam)
IB: ERG (santa cruz)
IB: SPOP
IB: AR
IB: ERF
IB: Tubulin
LNCaP
C
shGFP
shERF
1 2 3
IB: ERF
IB: ERG
IB: Tubulin
PC3
D
shGFP
shERG
shSPOP
shSPOP+shERG
G0/G1 64.67 66.06 66.74 67.38
S 18.62 18.01 17.95 17.71
G2/M 16.71 15.93 15.31 14.91
E
shGFP
shERG
shSPOP
shSPOP+shERG
Cell number (X10^5)
Days in culture
F
shGFP
shERG
shSPOP
shSPOP+shERG
7-AAD
PE Annexin V
0.4 0.6 0.1
0.4 1.0 0.2
0.4 0.8 0.2
0.3 1.0 0.2
G
siCtrl
siERG
siSPOP
siERG+siSPOP
H
Relative invasion (%)
siCtrl
siERG
siSPOP
siERG+siSPOP
I
shGFP
shSPOP
Relative mRNA level
ADAMTS1
CXCR4
MMP9
OPN
ARHGDIB
J
shGFP
shSPOP
A B C
IB: MMP9
IB: ADAMTS1
IB: SPOP
IB: ERG
IB: Vinculin
DU145
K
Cullin 3
SPOP
Gene Fusion
SPOP mutation
ERG, DEK, NCOA3, TRIM24
ADAMTS1, CXCR4, OPN et al targets
Cell migration and invasion
Cullin 4A
DDB1
DET1
COP1
Gene Fusion
JUN ETV1, ETV4, ETV5
Tumor

Figure S2. SPOP-mediated ERG degradation does not affect apoptosis but displays different effects on proliferation in AR positive and negative cells (related to Figure 2)

A-C. Immunoblot (IB) analysis of whole cell lysates (WCL) derived from DU145 **(A)** and LNCaP **(B)** cells infected with control (shGFP) and SPOP (shSPOP) lentiviral shRNA vectors or PC3 **(C)** cells infected with control (shGFP) and ERF (shERF) lentiviral shRNA vectors. The infected cells were selected with 1 μg/ml puromycin for 72 hours to eliminate the non-infected cells before harvesting.
D. PC3 cells were infected with the shGFP, shERG, shSPOP or shSPOP+shERG lentiviral shRNA vectors and selected with 1 μg/ml puromycin for 72 hours before FACS analysis of the cell cycle.
E. The growth curve of cell lines generated in **(C)**.
F. PE/Annexin V assay was performed with the cell line in **(C)**.
G-H. Representative images of invasive DU145 cells transfected with indicated siRNA in Matrigel invasion assays (**G**) and quantification of invaded cells (**H**). Data was shown as mean ± SD for three independent experiments. *$p < 0.05$, **$p < 0.001$, *t*-test.
I. Real-time PCR analysis to examine the mRNA levels of various ERG target genes upon depletion of SPOP. Data was shown as mean ± SD from three independent experiments.
J. IB analysis of WCL derived from DU145 cells infected with control (shGFP) and SPOP (shSPOP) lentiviral shRNA vectors. The infected cells were selected with 1 μg/ml puromycin for 72 hours to eliminate the non-infected cells before harvesting.
K. A schematic illustration of the proposed model by which ETV-fusion proteins evade Cullin 4/COP1-dependent ubiquitination and degradation pathway, while either mutation in the SPOP E3 ligase or ERG fusion proteins escape Cullin 3/SPOP-mediated ubiquitination and subsequent degradation of the ERG oncoprotein.

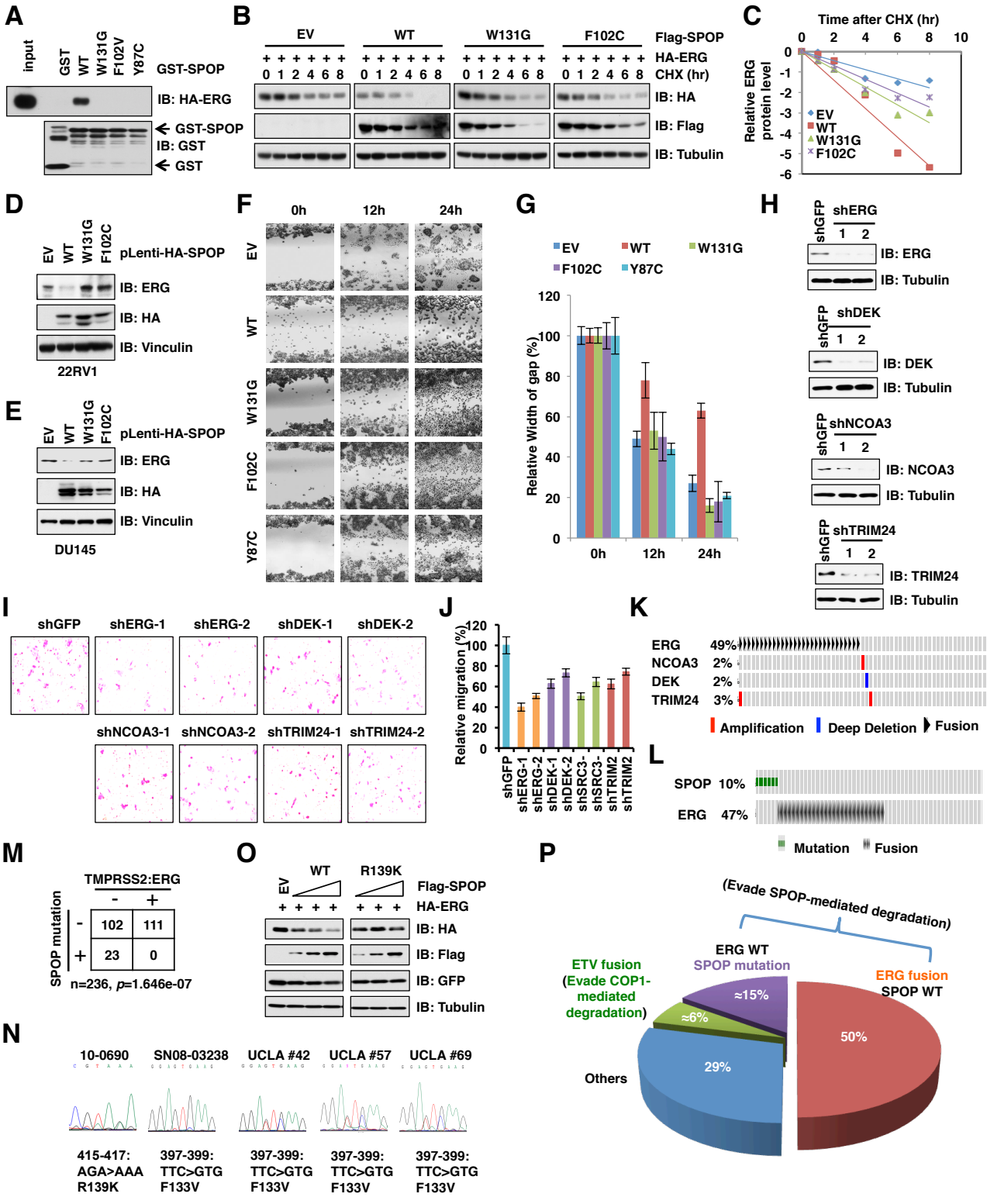
A
input
GST
WT
W131G
F102V
Y87C
GST-SPOP
IB: HA-ERG
GST-SPOP
IB: GST
GST
B
EV
WT
W131G
F102C
Flag-SPOP
HA-ERG
0 1 2 4 6 8
CHX (hr)
IB: HA
IB: Flag
IB: Tubulin
C
Time after CHX (hr)
0 2 4 6 8 10
Relative ERG protein level
0 -1 -2 -3 -4 -5 -6
EV
WT
W131G
F102C
D
EV
WT
W131G
F102C
pLenti-HA-SPOP
IB: ERG
IB: HA
IB: Vinculin
22RV1
E
EV
WT
W131G
F102C
pLenti-HA-SPOP
IB: ERG
IB: HA
IB: Vinculin
DU145
F
0h
12h
24h
EV
WT
W131G
F102C
Y87C
G
EV
WT
W131G
F102C
Y87C
Relative Width of gap (%)
120 100 80 60 40 20 0
0h
12h
24h
H
shGFP
shERG
1 2
IB: ERG
IB: Tubulin
shGFP
shDEK
1 2
IB: DEK
IB: Tubulin
shGFP
shNCOA3
1 2
IB: NCOA3
IB: Tubulin
shGFP
shTRIM24
1 2
IB: TRIM24
IB: Tubulin
I
shGFP
shERG-1
shERG-2
shDEK-1
shDEK-2
shNCOA3-1
shNCOA3-2
shTRIM24-1
shTRIM24-2
J
Relative migration (%)
120 100 80 60 40 20 0
shGFP
shERG-1
shERG-2
shDEK-1
shDEK-2
shSRC3-
shSRC3-
shTRIM2
shTRIM2
K
ERG 49%
NCOA3 2%
DEK 2%
TRIM24 3%
Amplification
Deep Deletion
Fusion
L
SPOP 10%
ERG 47%
Mutation
Fusion
M
TMPRSS2:ERG
- +
SPOP mutation
- 102 111
+ 23 0
n=236, p=1.646e-07
O
EV
WT
R139K
Flag-SPOP
HA-ERG
IB: HA
IB: Flag
IB: GFP
IB: Tubulin
P
(Evade SPOP-mediated degradation)
ERG WT
SPOP mutation
≈15%
ERG fusion
SPOP WT
50%
ETV fusion
(Evade COP1-mediated degradation)
≈6%
29%
Others
N
10-0690
SN08-03238
UCLA #42
UCLA #57
UCLA #69
415-417:
AGA>AAA
R139K
397-399:
TTC>GTG
F133V
397-399:
TTC>GTG
F133V
397-399:
TTC>GTG
F133V
397-399:
TTC>GTG
F133V

Figure S3. Prostate cancer-associated SPOP mutants are deficient in controlling ERG stability (related to Figure 3)

A. GST-pull down assay showed that prostate cancer-associated SPOP mutants fail to interact with ERG.

B. 293T cells transfected with HA-ERG and indicated Flag-SPOP constructs. 36 hours post-transfection, cells were treated with 100 μg/ml cycloheximide (CHX) for the indicated time period before harvesting. Immunoblot (IB) analysis of whole cell lysates (WCL) harvested at indicated time points.

C. Quantification of the band intensities in **(B)**. ERG bands were normalized to Tubulin, then normalized to the t = 0 time point.

D-E. IB analysis of WCL derived from 22RV1 (**D**) and DU145 (**E**) cells infected with control (EV) and indicated SPOP expressing vectors. The infected cells were selected with 200 μg/ml hygromycin for 72 hours to eliminate the non-infected cells before harvesting.

F-G. *In vitro* scratch assays were performed with cell lines in **(E).** Cells were seeded on 60 mm dishes and scratched on the surface with a pipette tip. Representative photographs at time points 0, 12 and 24 hours after the scratch **(F)**. Data was shown as mean ± SD for three independent experiments **(G)**.

H. IB analysis of WCL derived from DU145 cells stably expressing the SPOP-F102C mutant that were infected with control (shGFP) and ERG (shERG), DEK (shDEK), NCOA3 (shNCOA3), TRIM24 (shTRIM24) lentiviral shRNA vectors. The infected cells were selected with 1 μg/ml puromycin for 72 hours to eliminate the non-infected cells before harvesting.

I-J. Representative images of migrated DU145 cell lines generated in **(H)** in migration assay **(I)** and quantification of migrated cells **(J)**. Data was shown as mean ± SD for three independent experiments.

K. Bioinformatics analysis results from cBio (a database maintained by Memorial Sloan-Kettering) showed the frequency of ERG fusion, DEK, NCOA3, and TRIM24 mutation or amplification events in prostate cancer.

L. Bioinformatics analysis results from cBio showed SPOP mutation and ERG fusion are mutually exclusive in prostate cancer.

M. SPOP and ERG mutual exclusivity analysis on all of TCGA prostate cancer samples (236 patients).

N. Results of sequencing for five cases without ERG fusion.

O. IB analysis of WCL derived from 293T cells transfected with control (EV) and Flag-SPOP-WT, R139K (novel SPOP mutation identified by Sanger sequencing analysis in patient samples).

P. Schematic illustration to demonstrate that only approximately 6% of prostrate cancer cases possesses ETV fusions to evade the Cullin 4/COP1 destruction pathway. On the other hand, over 50% of prostate cases exhibit ERG fusion products that escape Cullin 3/SPOP-mediated degradation. Moreover, mutations in the SPOP tumor suppressor also lead to ERG accumulation in roughly 15% of prostate cancers. Altogether, aberrant elevation of the ERG oncoprotein by either mutations in the E3 ligase SPOP, or masking the SPOP recognizable degrons in the ERG fusion product, represents a major oncogenic event to facilitate prostate tumorigenesis

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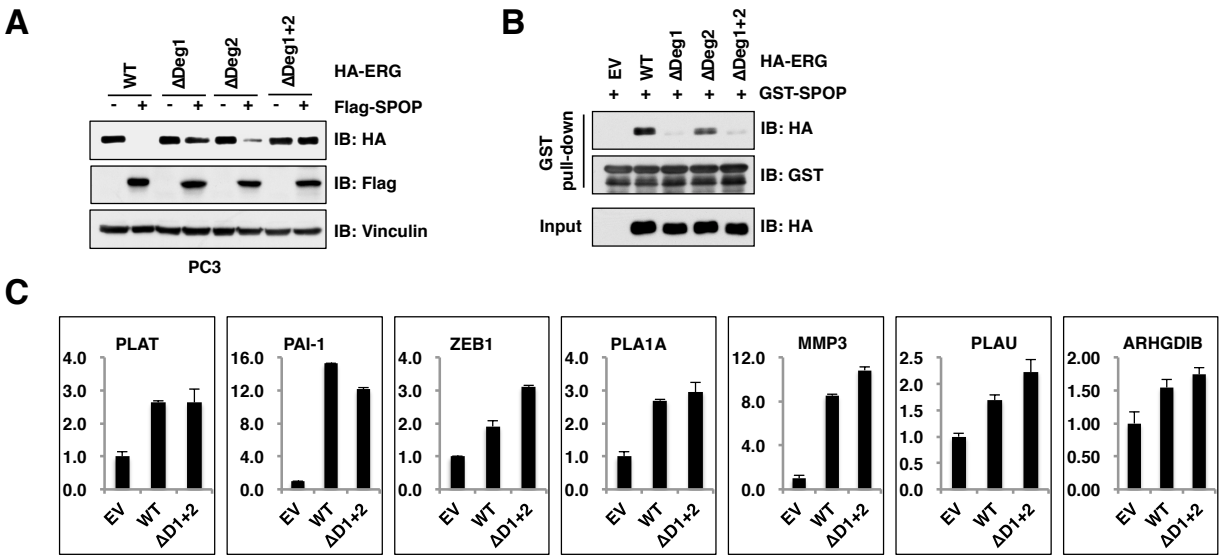

Figure S4. Deletion of the degron 1 blocks the SPOP/ERG interaction and ERG degradation (related to Figure 4)

A. Immunoblot (IB) analysis of whole cell lysates (WCL) derived from PC3 cells transfected with indicated HA-ERG and Flag-SPOP mammalian expressing constructs.
B. GST pull-down assays to demonstrate the reduced interaction of degron 1 or degron 1+2 deleted SPOP mutants.
C. Real-time PCR analyses to examine the mRNA levels of various indicated ERG target genes upon ectopic expression of ERG-WT and ΔDeg1+2 (ΔD1+2) in PC3 cells. Data was shown as mean ± SD from three independent experiments.

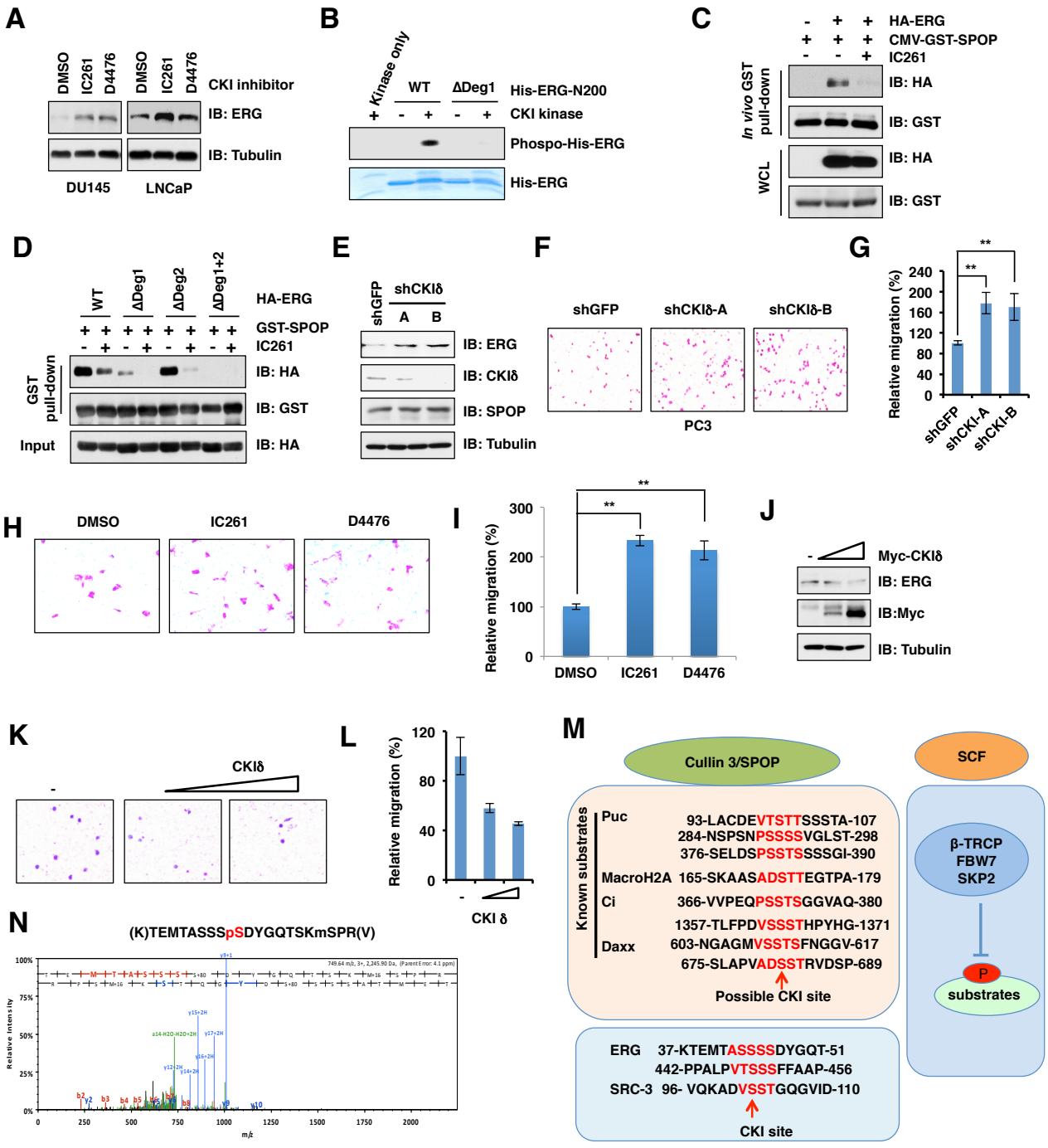
A
DMSO
IC261
D4476
DMSO
IC261
D4476
CKI inhibitor
IB: ERG
IB: Tubulin
DU145
LNCaP
B
Kinase only
WT
ΔDeg1
His-ERG-N200
CKI kinase
Phospo-His-ERG
His-ERG
C
HA-ERG
CMV-GST-SPOP
IC261
In vivo GST pull-down
IB: HA
IB: GST
WCL
IB: HA
IB: GST
D
WT
ΔDeg1
ΔDeg2
ΔDeg1+2
HA-ERG
GST-SPOP
IC261
GST pull-down
IB: HA
IB: GST
Input
IB: HA
E
shGFP
shCKIδ
A
B
IB: ERG
IB: CKIδ
IB: SPOP
IB: Tubulin
F
shGFP
shCKIδ-A
shCKIδ-B
PC3
G
Relative migration (%)
240
200
160
120
80
40
0
**
**
shGFP
shCKI-A
shCKI-B
H
DMSO
IC261
D4476
I
Relative migration (%)
300
200
100
0
**
**
DMSO
IC261
D4476
J
Myc-CKIδ
IB: ERG
IB:Myc
IB: Tubulin
K
CKIδ
-
L
Relative migration (%)
120
80
40
0
-
CKI δ
M
Cullin 3/SPOP
SCF
Known substrates
Puc
93-LACDEVTSTTSSSTA-107
284-NSPSNPSSSSVGLST-298
376-SELDSPSSTSSSGI-390
MacroH2A
165-SKAASADSTTEGTPA-179
Ci
366-VVPEQPSSTSGGVAQ-380
Daxx
1357-TLFPDVSSSTHPYHG-1371
603-NGAGMVSSTSFNGGV-617
675-SLAPVADSSTRVDSP-689
Possible CKI site
β-TRCP
FBW7
SKP2
P
substrates
ERG
37-KTEMTASSSSDYGQT-51
442-PPALPVTSSSFFAAP-456
SRC-3
96- VQKADVSSTGQGVID-110
CKI site
N
(K)TEMTASSSpSDYGQTSKmSPR(V)
Relative intensity
100%
75%
50%
25%
0%
0
250
500
750
1000
1250
1500
1750
2000
m/z

Figure S5. CKI is involved in ERG destruction (related to Figure 5)

A. Immunoblot (IB) analysis of whole cell lysates (WCL) derived from DU145 and LNCaP cells treated with CKI inhibitor IC261 or D4476 for 10 hours before harvesting.

B. *In vitro* kinase assay to demonstrate the degron 1 deleted ERG mutant cannot be phosphotated by CKI.

C-D. *In vivo* **(C)** and *in vitro* **(D)** GST-pull down assays to demonstrate the CKI inhibitor IC261 decreased ERG and SPOP interaction.

E. IB analysis of WCL derived from PC3 cells infected with control (shGFP) and CKIδ (shCKIδ) lentiviral shRNA vectors. The infected cells were selected with 1 μg/ml puromycin for 72 hours to eliminate the non-infected cells before harvesting.

F-G. Representative images of migrated shCKIδ cells in migration assay **(F)** and quantification of migrated cells **(G)**. Data was shown as mean ± SD for three independent experiments. $^{**}P < 0.001$, *t*-test.

H-I. Representative images of migrated DU145 cells treated with the indicated CKI inhibitor in migration assays **(H)** and quantification of migrated cells **(I)**. Data was shown as mean ± SD for three independent experiments. $^{**}P < 0.001$, *t*-test.

J. IB analysis of WCL derived from PC3 transfected with different dose of CKIδ expressing constructs.

K-L. Representative images of migrated PC3 transfected with different dose of CKIδ expressing constructs in migration assay **(K)** and quantification of migrated cells **(L)**. Data was shown as mean ± SD for three independent experiments. $^{**}P < 0.001$, *t*-test.

M. Like most of SCF type of E3 ligases that recognize substrates only after they are properly phosphorylated, our results indicate that like SRC-3, the interaction between ERG and Cullin 3-based SPOP E3 ligase could also be facilitated by CKI-mediated phosphorylation of the Ser/Thr residues within the identified degron. However, further studies are required to understand whether similar phosphorylation-induced interaction also exists for other identified SPOP substrates as all of them contain a stretch of Ser/Thr residues in their degron, which are predicted to be putative CKI or CKII phosphorylation sites.

N. The MS/MS fragmentation spectrum showing distinct **b-** (N-terminal) and **y-** (C-terminal) series fragment ions for the ERG peptide TEMTASSS**pS**DYGQTSK defining the pSer site in the degron region.

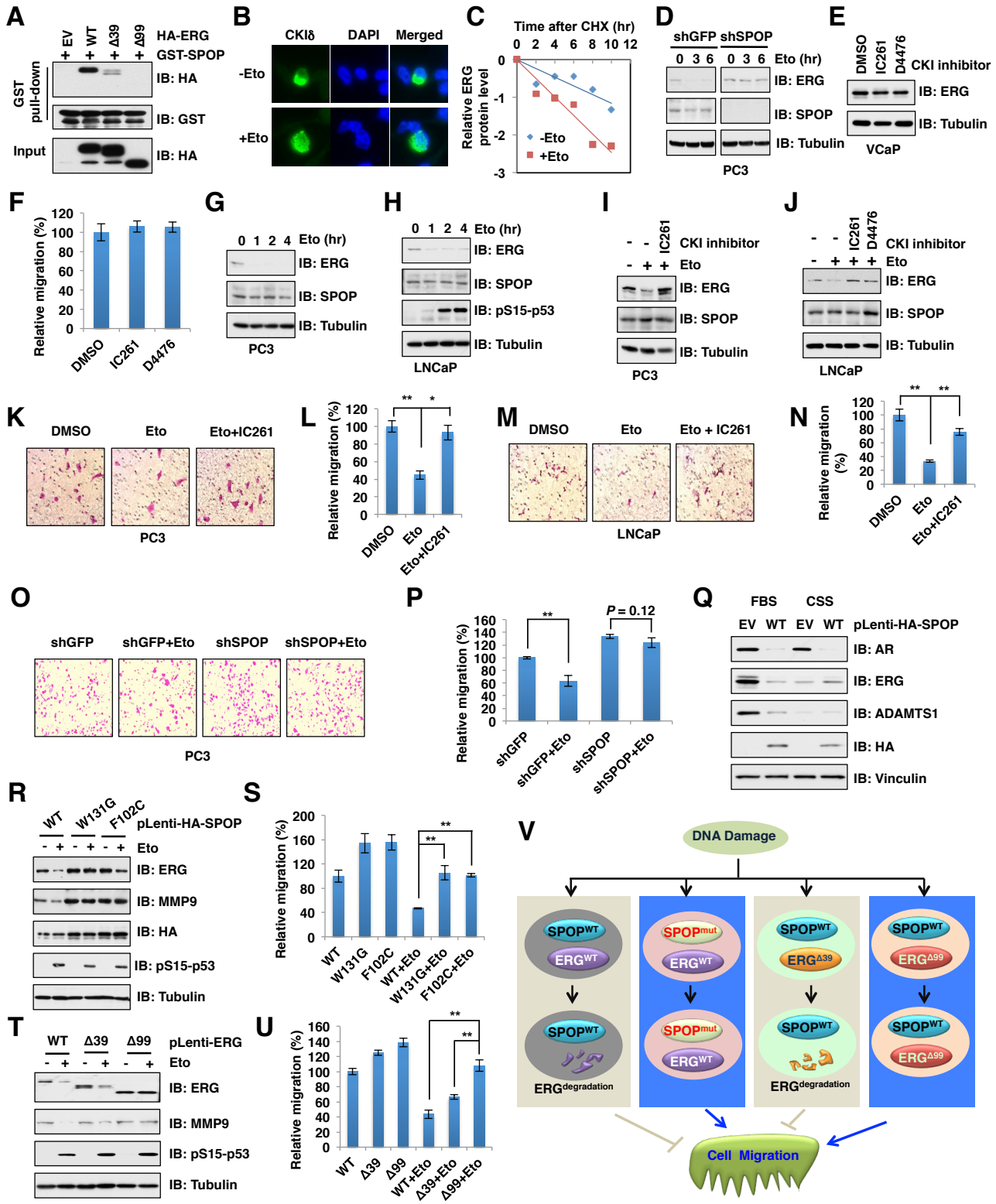
A
EV
WT
Δ39
Δ99
HA-ERG
GST-SPOP
GST pull-down
IB: HA
IB: GST
Input
IB: HA
B
CKIδ
DAPI
Merged
-Eto
+Eto
C
Time after CHX (hr)
0 2 4 6 8 10 12
Relative ERG protein level
0
-1
-2
-3
-Eto
+Eto
D
shGFP shSPOP
0 3 6 0 3 6 Eto (hr)
IB: ERG
IB: SPOP
IB: Tubulin
PC3
E
DMSO
IC261
D4476
CKI inhibitor
IB: ERG
IB: Tubulin
VCaP
F
Relative migration (%)
120
100
80
60
40
20
0
DMSO
IC261
D4476
G
0 1 2 4 Eto (hr)
IB: ERG
IB: SPOP
IB: Tubulin
PC3
H
0 1 2 4 Eto (hr)
IB: ERG
IB: SPOP
IB: pS15-p53
IB: Tubulin
LNCaP
I
IC261
CKI inhibitor
Eto
IB: ERG
IB: SPOP
IB: Tubulin
PC3
J
IC261
D4476
CKI inhibitor
Eto
IB: ERG
IB: SPOP
IB: Tubulin
LNCaP
K
DMSO
Eto
Eto+IC261
PC3
L
Relative migration (%)
**
*
DMSO
Eto
Eto+IC261
M
DMSO
Eto
Eto + IC261
LNCaP
N
Relative migration (%)
**
**
DMSO
Eto
Eto+IC261
O
shGFP
shGFP+Eto
shSPOP
shSPOP+Eto
PC3
P
Relative migration (%)
**
P = 0.12
shGFP
shGFP+Eto
shSPOP
shSPOP+Eto
Q
FBS
CSS
EV WT EV WT pLenti-HA-SPOP
IB: AR
IB: ERG
IB: ADAMTS1
IB: HA
IB: Vinculin
R
WT
W131G
F102C
pLenti-HA-SPOP
Eto
IB: ERG
IB: MMP9
IB: HA
IB: pS15-p53
IB: Tubulin
S
Relative migration (%)
**
**
WT
W131G
F102C
WT+Eto
W131G+Eto
F102C+Eto
T
WT
Δ39
Δ99
pLenti-ERG
Eto
IB: ERG
IB: MMP9
IB: pS15-p53
IB: Tubulin
U
Relative migration (%)
**
**
WT
Δ39
Δ99
WT+Eto
Δ39+Eto
Δ99+Eto
V
DNA Damage
SPOPWT
ERGWT
SPOPmut
ERGWT
SPOPWT
ERGΔ39
SPOPWT
ERGΔ99
SPOPWT
ERGdegradation
SPOPmut
ERGWT
SPOPWT
ERGdegradation
SPOPWT
ERGΔ99
Cell Migration

Figure S6. Etoposide-induced ERG degradation is dependent on SPOP and CKIδ (related to Figure 6)

A. *In vitro* GST-pull down assay to demonstrate that ERG fusion proteins decrease interaction with SPOP.
B. Etoposide (Eto) promoted CKIδ nuclear accumulation. Immunofluorescence and 4,6-diamidino-2-phenylindole (DAPI) staining of PC3 cells transfected with Myc-tagged CKIδ. 20 μM Eto was added for 3 hours.
C. Quantification of the band intensities in **(Figure 6M)**. ERG bands were normalized to Tubulin, then normalized to the t = 0 time point.
D. PC3 cells were infected with control (shGFP) and SPOP (shSPOP) lentiviral shRNA vectors. The infected cells were selected by 1 μg/ml puromycin for 72 hours to eliminate the non-infected cells. Afterwards, the indicated cell lines were treated with 20 μM Eto. At the indicated time points, WCL were prepared and immunoblots were probed with the indicated antibodies.
E. Immunoblot (IB) analysis of whole cell lysates (WCL) derived from VCaP cells treated with CKI inhibitor IC261 or D4476 for 10 hours before harvesting.
F. Quantification of migrated DU145 cells treated with the indicated CKI inhibitor. Data was shown as mean ± SD for three independent experiments.
G-H. IB analysis of PC3 **(G)** and LNCap **(H)** cells treated with 20 μM Eto for indicated time before harvesting.
I-J. IB analysis of WCL derived from PC3 **(I)** and LNCap **(J)** cells treated with 20 μM Eto and CKI inhibitors.
K-N. Representative images of migrated PC3 **(K)** or LNCaP **(M)** cells treated with Eto or together with CKI inhibitor (IC261) in migration assay and quantification of migrated cells PC3 **(L)** and LNCaP **(N)**. Data was shown as mean ± SD for three independent experiments. $*p < 0.05$, $**p < 0.001$, *t*-test.
O-P. Representative images of migrated depletion of SPOP cells treated with Eto in migration assay **(O)** and quantification of migrated cells **(P)**. Data was shown as mean ± SD for three independent experiments. $**P < 0.001$, *t*-test.
Q. IB analysis of WCL derived from VCaP cells stably expressing control (EV) or SPOP-WT (WT) cultured in normal FBS or charcoal stripped FBS (CSS) for 4 days before harvesting.
R. IB analysis of WCL derived from DU145 cells infected with control (EV) or the indicated SPOP expressing vectors. The infected cells were selected with 200 μg/ml hygromycin for 72 hours to eliminate the non-infected cells. Cells were treated with 20μM Eto for 4 hours before harvesting.
S. Quantification of migrated DU145 cell lines generated in **(R)** treated with 20 μM Eto for 4 hours. Data was shown as mean ± SD for three independent experiments. $**p < 0.001$
T. IB analysis of WCL derived from DU145 cells infected with control (EV) and the indicated ERG expressing vectors. Infected cells were selected with 1 μg/ml puromycin for 72 hours to eliminate the non-infected cells. Cells were treated with 20 μM Eto for 4 hours before harvesting.
U. Quantification of migrated DU145 cell lines generated in **(T)** treated with 20 μM Eto for 4 hours. Data was shown as mean ± SD for three independent experiments. $**p < 0.001$
V. Schematic illustration to demonstrate how the SPOP and ERG genetic status affect cell migration upon DNA damaging agent treatment.

Table S1. Primers for real-time PCR (related to Figure 2, S2 and S4)

ERG	Sense, 5'- CGTGCCAGCAGATCCTACG-3' Anti-sense, 5'- GGTGAGCCTCTGGAAGTCG-3'
SPOP	Sense, 5'-GCCCTCTGCAGTAACCTGTC-3' Anti-sense, 5'-GTCTCCAAGACATCCGAAGC-3'
ADAMTS1	Sense, 5'-ACTGGAAGCATAAGAAAGAAGCG-3' Anti-sense, 5'-AATTCTGCCATCGACTGGTCT-3'
CXCR4	Sense, 5'-ACTACACCGAGGAAATGGGCT-3' Anti-sense, 5'-CCCACAATGCCAGTTAAGAAGA-3'
MMP9	Sense, 5'-TGTACCGCTATGGTTACACTCG-3' Anti-sense, 5'-GGCAGGGACAGTTGCTTCT-3'
OPN	Sense, 5'-CTCCATTGACTCGAACGACTC-3' Anti-sense, 5'-CAGGTCTGCGAAACTTCTTAGAT-3'
ARHGDIB	Sense, 5'-GACTGGGGTGAAAGTGGATAAAG-3' Anti-sense, 5'-TCGTCGGTGAAGAAGGACTTG-3
PLAT	Sense, 5'-AAACCCAGATCGAGACTCAAAGC-3' Anti-sense, 5'-GGTAGGCTGACCCATTCCC-3'
PAI-1	Sense, 5'-AGTGGACTTTTCAGAGGTGGA-3' Anti-sense, 5'-GCCGTTGAAGTAGAGGGCATT-3
ZEB1	Sense, 5'-GATGATGAATGCGAGTCAGATGC-3' Anti-sense, 5'-ACAGCAGTGTCTTGTTGTTGT-3
PLA1A	Sense, 5'-GGACGCTGTCTGGATTGCTTT-3' Anti-sense, 5'-CGGCTCTATCTTGACACCACC-3
MMP3	Sense, 5'-AGTCTTCCAATCCTACTGTTGCT-3' Anti-sense, 5'-TCCCCGTCACCTCCAATCC-3'
PLAU	Sense, 5'-GCTTGTCCAAGAGTGCATGGT-3' Anti-sense, 5'-CAGGGCTGGTTCTCGATGG-3'

Supplemental Experimental Procedures

Cell culture

HEK293T, DU145 and VCap cells were maintained in DMEM medium supplemented with 10% FBS. PC3 and LNCap cells were cultured in RPMI 1640 medium with 10% FBS. Cell transfection was performed as described previously (Wei et al., 2004). Lentiviral shRNA virus packaging and subsequent infection of various cell lines were performed according to the protocol described previously (Boehm et al., 2005). CKI inhibitor IC261 (Calbiochem, 400090) and D4476 (Sigma, D1994), etoposide (Sigma, E1383) were used at the dose as indicated. Cycloheximide (CHX) assays were performed as described previously (Sarbassov et al., 2005).

Plasmids

HA-ERG, HA-ERF, HA-ERG-Δ39, HA-ERG-Δ99, HA-ETV1, HA-ETV4 and HA-ETV5 were constructed by sub-cloning the corresponding cDNAs into pcDNA3-HA vector. Flag-SPOP construct was obtained from Dr. Pengbo Zhou. pCMV-GST-SPOP and pGEX-SPOP were generated by sub-cloning SPOP cDNAs into pCMV-GST and pGEX-4T-1 vectors, respectively. Flag-COP1, Myc-COP1 and Flag-DET1 constructs were kind gift from Dr. William Kaelin. Flag-Keap1 was purchased from Addgene. Isoforms of Myc-CKI, Myc-CKIIα, Myc-CKIIβ, Myc-Cullins constructs and shRNA constructs against GFP and CKIδ were described previously (Inuzuka et al., 2010). pLenti-HA-SPOP was constructed by sub-cloning the SPOP cDNA into pLenti-HA-hygro vector**.** Various SPOP and ERG mutants were generated using the QuikChange XL Site-Directed Mutagenesis Kit (Stratagene) according to the manufacturer's instructions. Details of plasmid constructions are provided upon request.

shRNAs

shRNA vectors to deplete endogenous ERG, SPOP, DEK, NCOA3, TRIM24 were purchased from Sigma. siControl (sense, 5'-GAAAAACUCAUAUAAAUCGCGCCAC-3'). siERG (sense, 5'-AGAGACAAUC GAUAUAAUGUGGCCT-3'). siSPOP (sense, 5'-GGCUCACAAGGCUAUCUUAGCAGCT-3'). siRNA oligos were transfected into cells with Dharmafect (Thermo Fisher Scientific) according to the manufacturer's instruction.

Antibodies

All antibodies were used at a 1:1000 dilution in 5% non-fat milk for western blot. Anti-ERG antibody [EPR3864 (2)] and (C-20) were purchased from Abacm and Santa Cruz, respectively. Anti-SPOP antibody (16750-1-AP) was purchased from Proteintech. Anti-Cul 3 (2759), anti-GST (2625), polyclonal anti-Myc-Tag antibody (2278) and monoclonal anti-Myc-Tag (2276) antibodies were purchased from Cell Signaling. Polyclonal anti-HA antibody (SC-805), anti-p27 (SC-527) and anti-CyclinE (SC-247) antibodies were purchased from Santa Cruz. Polyclonal anti-Flag antibody (F-2425), monoclonal anti-Flag antibody (F-3165, clone M2), anti-Tubulin antibody (T-5168), anti-Vinculin antibody (V-4505), anti-Flag agarose beads (A-2220), anti-HA agarose beads (A-2095), peroxidase-conjugated anti-mouse secondary antibody (A-4416) and peroxidase-conjugated anti-rabbit secondary antibody (A-4914) were purchased from Sigma. Monoclonal anti-HA antibody (MMS-101P) was purchased from Covance. Anti-GFP (8371-2) antibody was purchased from Clontech.

Immunoblots and immunoprecipitation

Cells were lysed in EBC buffer (50 mM Tris pH 7.5, 120 mM NaCl, 0.5% NP-40) supplemented with protease inhibitors (Complete Mini, Roche) and phosphatase inhibitors (phosphatase inhibitor cocktail set I and II, Calbiochem). The protein concentrations of lysates were measured by the Beckman Coulter DU-800 spectrophotometer using the Bio-Rad protein assay reagent. Same amounts of whole cell lysates were

resolved by SDS-PAGE and immunoblotted with indicated antibodies. For immunoprecipitation, 1000 μg lysates were incubated with the indicated antibody (1-2 μg) for 3-4 hours at 4 °C followed by 1 hour incubation with Protein A sepharose beads (GE Healthcare). Immunoprecipitants were washed five times with NETN buffer (20 mM Tris, pH 8.0, 100 mM NaCl, 1 mM EDTA and 0.5% NP-40) before being resolved by SDS-PAGE and immunoblotted with indicated antibodies. Quantification of the immunoblot band intensity was performed with ImageJ software.

In vitro ubiquitination assay

In vitro ubiquitination assay was performed as described previously (Inuzuka et al., 2010). Briefly, 293T cells were transfected with pCMV-GST-SPOP, Myc-Cul 3 and Myc-Rbx1 to purify SPOP/Cullin 3/Rbx1 complex by GST affinity precipitation. GST-ERG-N200 protein (wild-type and ΔDeg1) was incubated with purified SPOP/Cullin 3/Rbx1 complex together with E1, E2 (UbcH5a and UbcH3) and ubiquitin. The reactions were stopped by SDS sample buffer and resolved by SDS-PAGE for immunobloting.

Bioinformatics analysis

Spearman correlation analysis gene expression changes in paired normal and tumor specimens in SPOP mutant prostate tumors and ERG fusion positive prostate tumors (Rho=0.64, $p < 2.2e-16$). Each point in the scatterplot is a gene, and the x- and y- axes are the significance analysis of microarrays D-statistic for SPOP mutant and ERG fusion samples, respectively. The D-statistic indicates the gene's direction and magnitude of differential expression in the paired tumor versus normal sample. Values in red are significantly associated with tumor (FDR<5%) in SPOP mutation and ERG fusion samples.

Mass spectrometry analysis to detect ERG S46 phosphorylation in cells

The procedure was performed as described previously (Inuzuka et al., 2010) with minor modifications. Briefly, 293T cells were transiently co-transfected with HA-ERG and Myc-CKI. 48 hours post-transfection, cells were harvested with EBC buffer and the whole-cell lysates were collected to perform HA immunoprecipitation. The HA immunoprecipitates were then resolved on SDS-PAGE and visualized by colloidal coomassie blue. The band containing HA-ERG was excised and digested with trypsin. The peptide mixture was analyzed using C_{18} microcapillary liquid chromatography tandem mass spectrometry (LC-MS/MS) using a high resolution hybrid Orbitrap Elite mass spectrometer (Thermo Scientific) coupled to an EASY-nLC II nanoflow HPLC (Thermo Scientific). Collision induced dissociation (CID) fragmentation spectra was searched against the concatenated decoy Human protein database (UniProt) using the Mascot 2.5 search engine. Data were interpreted and reported using Scaffold 4.4 and Scaffold PTM 2.1.3 software (Proteome Software). Peptides were accepted if they passed a 1.0% false discovery rate (FDR) threshold.

RNA extraction and SPOP sequencing

To isolate total RNA from formalin-fixed and paraffin embedded tissues, 10 μm sections were collected on charged slides, and the tissue areas containing cancer and normal prostate, respectively, were circled based on histologic examination of the corresponding H&E-stained slides. Recoverall™ (Invitrogen, Carlsbad, CA) was used to isolate total RNA, followed by reverse-transcription by Superscript III (Invitrogen, Carlsbad, CA) through random priming and PCR amplification. SPOP primers (Exons 6 and 7): 5'-TTTGCGAGTAAACCCCAAAG-3' and 5'-CGCAGAAGAGGGTAAGCTTGT-3' were used for amplifying and sequencing SPOP.

Supplemental References

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